SUPPLEMENTAL TEXT

SUPPLEMENTAL MATERIALS AND METHODS

Cytotoxicity of FFAs on human sebocytes

To examine the effect of FFAs on the cell viability of the human SZ95 sebocytes, the cells (1×10^5/well) were incubated with LA, PA or OA (25 μg/ml) in 1% FBS-Sebomed® for 24 hr at 37°C. Control received an equal amount of DMSO (0.5% (v/v)). Triton X-100 (0.01%) was used to achieve 0% of cell viability. After incubation, cell viability of sebocytes was determined with acid phosphatase (ACP) assays (Martin and Clynes, 1991). Cells were washed with PBS three times and incubated with 100 μl of 10 mM *p*-nitrophenyl phosphate (*p*NPP) in ACP assay buffer (1 M sodium acetate buffer, pH=5.5, containing 0.1% (w/v) Triton X-100) for 1 h at 37°C. After that, 10 μl of 1N NaOH was added to stop the reaction and absorbance at 405 nm was measured. The cell viability was calculated as: (the OD_{405} difference between FFA treatment and Triton-X-100 treatment) ÷ (the OD_{405} difference between no treatment and Triton X-100 treatment) ×100 (%).

In vitro antimicrobial assay

To determine bactericidal effect of FFAs against *P. acnes*, *P. acnes* (1×10^6 CFU/ml) was incubated with LA, PA or OA at the various concentrations (0-100 μg/ml) in PBS on a 96-well

microplate (100 μl/well) at 37°C for 5 hr under anaerobic conditions. The control received an equal amount of DMSO (0.5% (v/v)). The reaction mixture was diluted 1:10-1:10^4 with PBS. CFU was determined by spotting 5 μl of the dilution on a Brucella broth agar plate.

SUPPLEMENTAL REFERENCE

Martin A, Clynes M (1991) Acid phosphatase: endpoint for in vitro toxicity tests. *In Vitro Cell Dev Biol* 27A:183-184.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Effect of FFAs on the cell viability of the human SZ95 sebocytes. The immortalized human SZ95 sebocytes (1×10^5 cells/well) were incubated with LA, PA or OA (25 μg/ml) in 1% FBS-Sebomed® for 24 hr at 37°C. As a background, Triton X-100 (0.1% (v/v)) was added to achieve 0% of cell cytotoxicity. After incubation, cell viability of sebocytes was determined with *p*NPPas described in SUPPLEMENTAL MATERIALS AND METHODS. Data represent mean ± SE of five individual experiments (*P<0.05 by Student's *t*-test).

Figure S2. Bactericidal effects of LA, PA and OA on *P. acnes*. *P. acnes* (1×10^6 CFU/ml) was incubated with 0-100 μg/ml of LA, PA or OA in 0.5% DMSO in PBS for 5 hr under anaerobic

conditions. After incubation, *P. acnes* suspension was diluted 1:10 -1:10^4 with PBS, and 5 μl of the dilutions was spotted on a Brucella Broth agar plate supplemented with 5% defibrinated sheep blood and hemin and vitamin K. After liquid in the *P. acnes* suspension was absorbed into the agar, the plate was incubated under anaerobic conditions to quantify CFU of *P. acnes*. Data represent mean ± SE of three individual experiments. UD: undetectable

Figure S3. Pre-incubation with anti-CD36 IgGs or BMS-345541 and subsequent incubation with FFAs did not affect the cell viability of the human SZ95 sebocytes.

(a-b) The effects of pre-treatment with anti-CD36 IgGs or BMS-345541 and subsequent incubation with FFAs on the cell viability of the SZ95 sebocytes were examined. The cells (2×10^6/well) cultured on a 24-well plate were preincubated with mouse anti-CD36 IgG (5 μg/ml) or normal mouse IgG in 1% FBS-Sebomed® (250 μl) for 2 hr at 37°C (a). The cells (2×10^6/well) cultured on a 24-well plate were preincubated with BMS-345541 (20 μM) or an equal amount of DMSO (0.1%) in 1% FBS-Sebomed® (250 μl) for 1 hr at 37°C (b). After preincubation, LA, PA or OA (25 μg/ml) was added to the cells, which were subsequently incubated for 24 hr at 37 °C. Control received an equal amount of DMSO (0.5% (v/v)). After incubation, cell viability of sebocytes was determined with *p*NPP as described in SUPPLEMENTAL MATERIALS AND METHODS. Data represent mean ± SE of six individual experiments.

Figure S4. Effect of FFAs on the hBD-2 expression in human keratinocytes.

The human keratinocyte cell line, HaCaT, (1×10^6/well) were incubated with LA, PA, or OA (25 μg/ml) in 1% FBS-DMEM containing 0.5% (w/v) DMSO for 24 hr at 37°C. The control received an equal amount of DMSO. mRNA expression of hBD-2 was evaluated by real-time qPCR, normalized to that of GAPDH, and then plotted as relative expression compared to vehicle-treated cells. Data represent mean ± SE of three individual experiments $^{**}P<0.01$, $^{***}P<0.005$ by Student's *t*-test, vs. vehicle control).

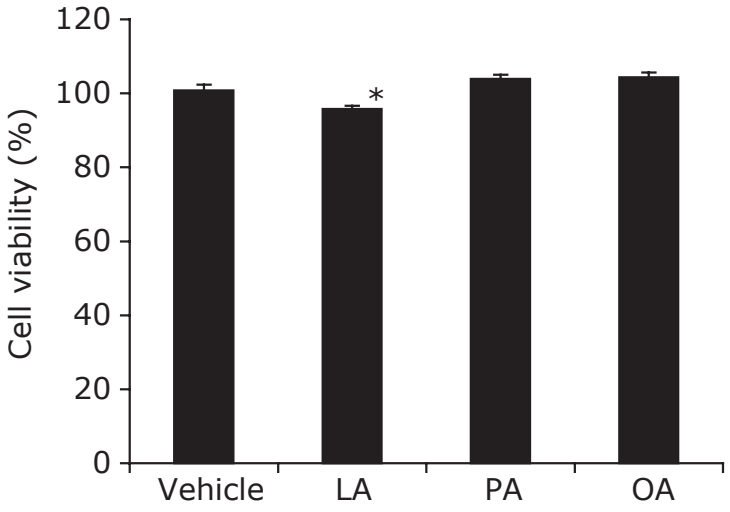


Figure S1

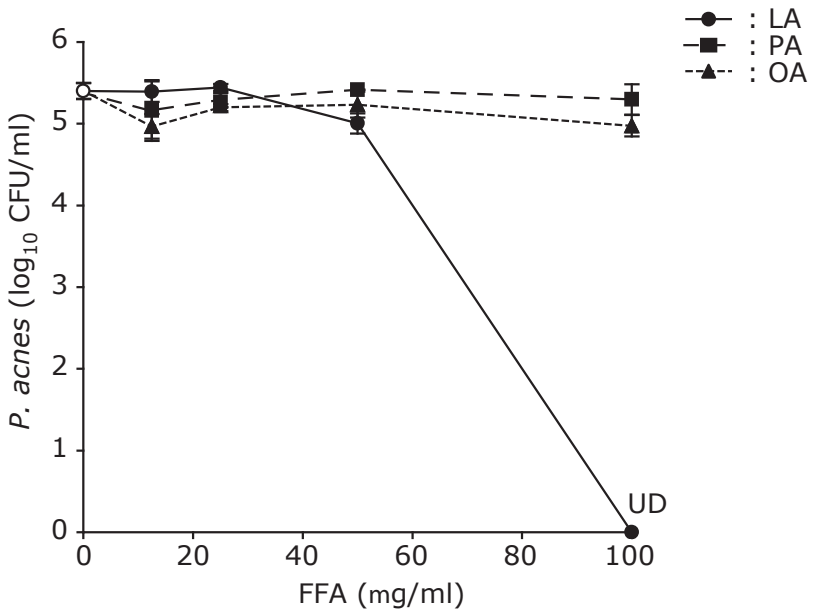

: LA
: PA
: OA
P. acnes (log10 CFU/ml)
FFA (mg/ml)
UD
0
1
2
3
4
5
6
0
20
40
60
80
100

Figure S2

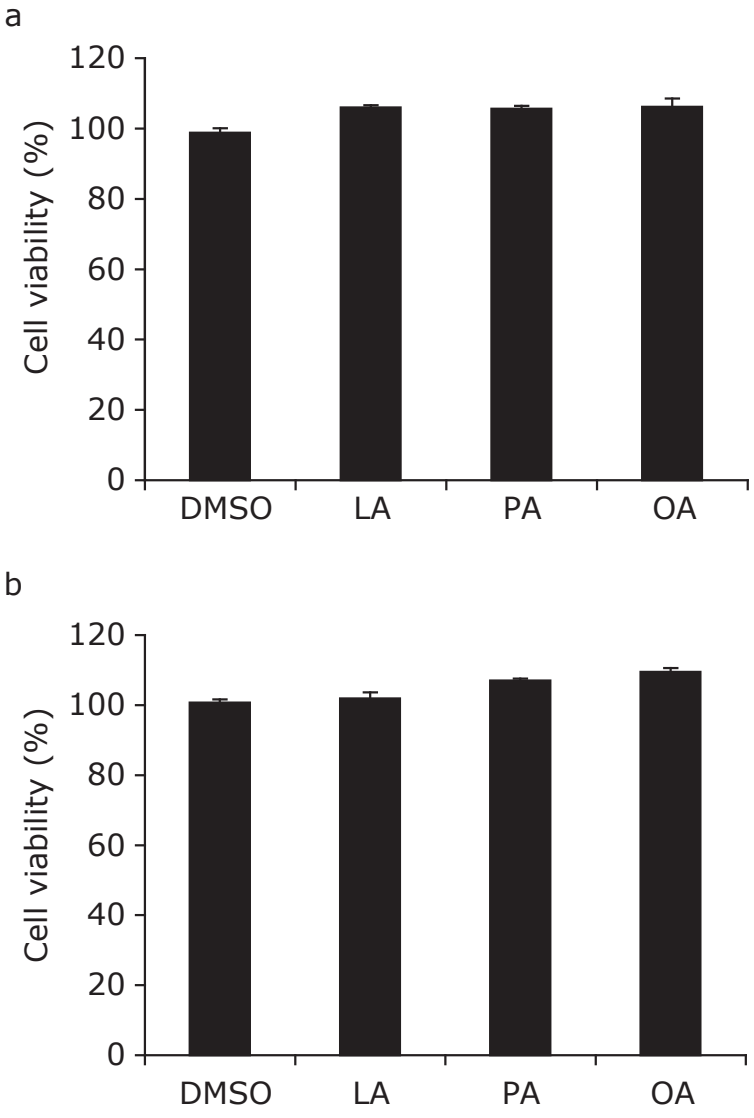
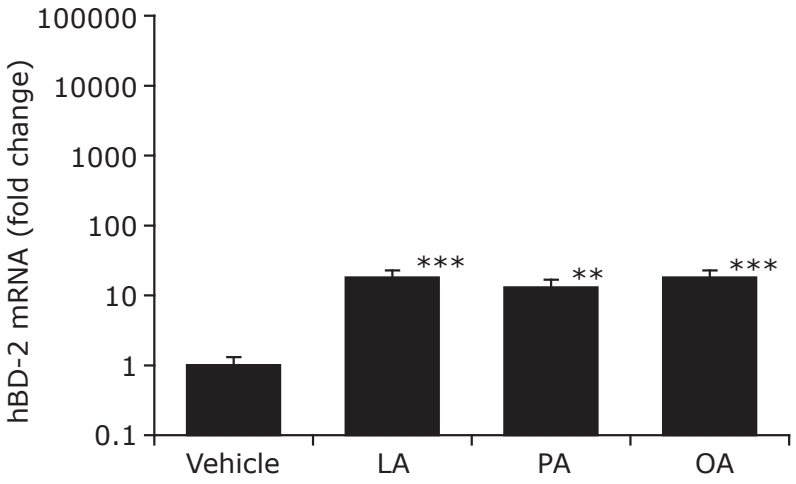

a
120
100
80
60
40
20
0
Cell viability (%)
DMSO
LA
PA
OA
b
120
100
80
60
40
20
0
Cell viability (%)
DMSO
LA
PA
OA

Figure S3


100000
10000
1000
100
10
1
0.1
hBD-2 mRNA (fold change)
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Vehicle
LA
PA
OA

Figure S4